ATLANTIS PRESS

Advances in Biological Sciences Research

Asmoro Lelono · Muhammad Akbar Bahar · Syubanul Wathon · Kartika Senjarini · Asep Ginanjar Arip · Ramdhan Putrasetya · Beny Andika · Nadhea Ayu Sukma · Bambang Sugiharto *Editors*

Proceedings of the 4th International Conference on Life Sciences and Biotechnology (ICOLIB 2021) · Volume 27

OPEN ACCESS

Advances in Biological Sciences Research 27

Series Editor

Wanshu Ma, *Northwestern University, Chicago, USA*

The proceedings series Advances in Biological Sciences Research aims to publish proceedings from conferences on the theories and methods in fields of biological sciences. Topics covered by this series: Biology, Biomedical sciences, Biochemistry, Genetics, Genomics, Molecular biology, Biophysics, Biotechnology, Cancer research, Cell biology, Clinical biochemistry, Developmental biology, Endocrinology, Molecular medicine, Physiology, Structural biology, Ageing, Bioinformatics, Agriculture, Agronomy, Crop science, Animal science, Zoology, Aquatic science, Ecology, Evolution, Behavior, Systematics, Food science, Forestry, Horticulture, Insect science, Plant science, Soil science.

Asmoro Lelono · Muhammad Akbar Bahar · Syubanul Wathon · Kartika Senjarini · Asep Ginanjar Arip · Ramdhan Putrasetya · Beny Andika · Nadhea Ayu Sukma · Bambang Sugiharto
Editors

Proceedings of the 4th International Conference on Life Sciences and Biotechnology (ICOLIB 2021)

ATLANTIS PRESS

ISSN 2731-7846 ISSN 2468-5747 (electronic)
Advances in Biological Sciences Research
ISBN 978-94-6463-061-9 ISBN 978-94-6463-062-6 (eBook)
https://doi.org/10.2991/978-94-6463-062-6

This Atlantis Press imprint is published by the registered company Atlantis Press International B.V., part of Springer Nature
The registered company address is: Van Godewijckstraat 30 3311 GX Dordrecht Netherlands

Preface ICOLIB 2021

I am pleased to welcome all of the speakers and participants to the 2021 International Conference on Life Sciences and Biotechnology (4th ICOLIB), which is held from 15 to 16 November 2021, virtually on Zoom meeting. The conference is organized by the Department of Biology, Faculty of Mathematics and Natural Sciences, the University of Jember.

This year the conference's theme is "Towards Sustainable Development: Application of Biosciences to Improve Welfare and Quality of Life". Along with the theme, we have four conference topics; there are Applied Sciences (Agriculture, Biotechnology and Bioinformatics), Basic Sciences (Ecology, Zoology, Botany, and Microbiology), Biodiversity and Bio-conservation, and Health and Medicine (Pharmacy and Medical Sciences). This scientific event provides a platform for researchers, academics, professionals, industries, and policymakers to exchange ideas, share the recent advances and development in life sciences, and can be a valuable place for starting fruitful collaboration, especially in uncovering the potential of biodiversity at the molecular level to biosphere.

This year's conference is also the first time held online due to the global pandemic situation. However, it becomes a blessing in disguise, because the conference becomes accessible to a wider audience and participants from all over the world. The number of participants registered is 223, among them, 170 participants will present their research.

Most of the participants are from Indonesia but also we have participants from Malaysia, Czech Republic, China, Philippines, Pakistan, and India. Besides the participants, the online of this conference also allows us to invite speakers from Netherlands, Germany, USA, and Australia. There will be Prof. A.G.G (Ton) Groothuis from GELIFES Institute, Groningen University Netherlands, Prof Antonius Suwanto from IPB Indonesia, Prof Simon Griffith from Department of Biological Sciences at Macquarie University, Sydney, Dr. Jorge A. Santiago-Blay from the Department of Paleobiology MRC-121 National Museum of Natural History Smithsonian Institution, USA, Prof. Elvira Hoerandl from George-August, Goothingen University Germany, Dr. Kahar Muzakar from Biology Department, Jember University, and Dr. Christina Bauch from Instituto Universitario in Lisbon, Portugal and Groningen University, Netherlands.

The output of this conference will be published in the Atlantis Press Proceeding: Part of Nature in series of "Advances in Biological Sciences Research", the Journal of ILMU Dasar MIPA, and the Journal of Tropical Biodiversity and Biotechnology. Finally, I would like to acknowledge the Board of Jember University which supported this conference. And I also like to thank to Vanadia and DAAD as one of the main sponsors which make this conference possible.

I hope this conference will be fruitful for everyone. We look forward to seeing you all at the next ICOLIB conference.

Asmoro Lelono
Chairman of 4th ICOLIB 2021

Organization

Chair Person

Asmoro Lelono	Zoology Laboratory, Biology Department, Mathematics and Natural Sciences Faculty, Jember UniversityBehavioural Department, GELIFES Institute, Groningen University

General Chairs

Esti Utarti	Microbiology Laboratory, Biology Department, Mathematics and Natural Sciences Faculty, Jember University, Indonesia
Ivan Surya Pradipta	Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Padjadjaran University

Technical Committee

Sutoyo	Microbiology Laboratory, Biology Department, Mathematics and Natural Sciences Faculty, Jember University
Fuad Bahrul Ulum	Botany Laboratory, Biology Department, Mathematics and Natural Sciences Faculty, Jember University

Steering Committee

Hari Sulistyowati	Ecology Laboratory, Biology Department, Mathematics and Natural Sciences Faculty, Jember University
Rike Oktarianti	Biotechnology Laboratory, Biology Department, Mathematics and Natural Sciences Faculty, Jember University
Sattya Arimurti	Microbiology Laboratory, Biology Department, Mathematics and Natural Sciences Faculty, Jember University

Organizing Committee

Purwatiningsih	Zoology Laboratory, Biology Department, Mathematics and Natural Sciences Faculty, Jember University
Mukh. Suudi	Botany Laboratory, Biology Department, Mathematics and Natural Sciences Faculty, Jember University

Chief Editor

Editorial Boards

Contents

Peer-Review Statements

Bambang Sugiharto[1,2(✉)], Asmoro Lelono[1,3], Muhammad Akbar Bahar[4,5], Syubanul Wathon[1], Asep Ginanjar Arip[6], Kartika Senjarini[1], Ramdhan Putrasetya[1], Beny Andika[1], and Nadhea Ayu Sukma[1]

[1] Department of Biology, Faculty of Mathematic and Natural Sciences, Jember University, Jember, East Java, Indonesia
sugiharto.fmipa@unej.ac.id
[2] Centres for Development of Advanced Science and Technology (CDAST), Jember University, Jember, East Java, Indonesia
[3] Behavioural Department, GELIFES Institute, Groningen Universities, Groningen, Netherlands
[4] Pharmacy Faculties, Hasanuddin University, Makassar, South Sulawesi 90245, Indonesia
[5] Institute of Clinical Pharmacy, Faculty of Pharmacy, University of Szeged, Szeged, Hungary
[6] Master of Biology Education, School of Postgraduate Study, Kuningan University, Kuningan, West Java, Indonesia

AQ1 All of the articles in this proceedings volume have been presented at the International Conference on Life Science and Biotechnology (ICOLIB) on November 15–16, 2021 at Jember University Indonesia. These articles have been peer-reviewed by the members of the Scientific Committee of ICOLIB and approved by the Editor-in-Chief, who affirms AQ2 that this document is a truthful description of the conference's review process.

1 Review Procedure

The reviews were double-blind. Each submission was examined by two reviewer(s) independently. The conference submission management system was easy chair.

We divided the submission of the ICOLIB participant into two categories, the first is abstract and the second the full manuscript submission. The submissions of the abstract were first screened for generic quality, relatedness to the main topic and suitableness by the editorial team. Based on this initial screening, all of the abstracts would be classified by the main theme, i.e. Applied Sciences (Agriculture, Biotechnology & Bioinformatics), Basic Sciences (Ecology, Zoology, Botany, and Microbiology), Biodiversity & Bioconservation, Health & Medicine (Pharmacy & Medical Sciences). All of the selected abstracts would be presented by their author during the ICOLIB conferences. The committee gave an option to the authors to publish their manuscripts or just presented their work in the ICOLIB conference.

All of the complete manuscripts then follow the review process, the first step was to evaluate the relatedness to the proceedings series "Advances in Biological Sciences Research", scientific quality, novelty and contribution to the science. The second step is checking the similarity using Turnitin to evaluate the textual overlap and detect the possible sign of plagiarism. The third step was to send for peer review by matching each

B. Sugiharto—Editors-in-Chief of the ICOLIB.

A. Lelono et al. (Eds.): ICOLIB 2021, ABSR 27, pp. 1–3, 2023.
https://doi.org/10.2991/978-94-6463-062-6_1

paper's topic with the reviewers' expertise, taking into account any competing interests. However, in some case, we also sent the manuscript to the third reviewer to consider another opinion if the first two reviewers has an opposite decision. A paper could only be considered for acceptance if it had received favourable comments and suggestions from the two reviewers. The recommendations then sent back to the author to address the reviewer's comment. The acceptance or rejection of a revised manuscript was final. In the final steps, all of the manuscripts were adjusted in their layout and some of the technical editing for the pre-print version. This preprint document would be sent to the author for clarification. They also should be sent a statement of the novelty and originality of the study.

2 Quality Criteria

Reviewers were instructed to assess the quality of submissions solely based on the academic merit of their content along the following dimensions. The editorial gave a rubric for a guideline which contains some important aspect related to the quality of the manuscript such as:

1. Pertinence of the article's content to the scope and themes of the conference;
2. Clear demonstration of originality, novelty, and timeliness of the research;
3. Soundness of the methods, analyses, and results;
4. Adherence to the ethical standards and codes of conduct relevant to the research field;
5. Clarity, style, cohesion, and accuracy in language and other modes of expression, including figures and tables.

We have a policy that each manuscript should be reviewed by two reviewers and each reviewer only reviews two manuscripts. The consequences of this policy is that we contact more reviewers, in total we ask 61 reviewer for completing the review process.

3 Key Metrics

Total submissions	118
Number of articles sent for peer review	67
Number of accepted articles	61.
Acceptance rate	51.5%
Number of reviewers	61

4 Competing Interests

Neither the Editor-in-Chief nor any member of the Scientific Committee declares any competing interest.

The Apyrase Functional Properties of the 56 kDa Protein from *Aedes aegypti* Salivary Gland

Rike Oktarianti, Alfan Suhardiansyah, Elisa Erni, Syubbanul Wathon, and Kartika Senjarini(✉)

Biology Department, Faculty of Mathematics and Natural Sciences, University of Jember, Jember 68121, Indonesia
senjarini@unej.ac.id

Abstract. Apyrase is an enzyme an inhibit platelet aggregation process, capable of degrading ADP in the process blood feeding and mostly found in hematophagous arthropods. While vector's blood feeding, this apyrase salivary protein is responsible for inhibiting platelet aggregation in the human host, by hydrolyzing adenosine diphosphate or adenosine triphosphate molecules that produce adenosine monophosphate thus decrease platelet aggregation. Our previous study reported that the immunogenic proteins 56 kDa from salivary gland of dengue's vector *Aedes aegypti* constituted high apyrase activity. This study wanted to analyze apyrase functional properties of this immunogenic protein. The amount of inorganic phosphate released from ADP degradation by apyrase was analyzed using by malachite green detection kit. We also further analyzed its platelet aggregation inhibition activity. The results showed that 56 kDa immunogenic protein has high apyrase activity with 33.30 nmol/well inorganic phosphate released, half of positive control activity (ATP-se) and it can inhibit platelet aggregation by in vitro was 40–50%.

Keywords: Apyrase · 56 kDa immunogenic protein · Aedes aegypti

1 Introduction

Apyrase is an enzyme mostly found in hematophagous arthropods, and is able to degrade adenosine diphosphate (ADP) and adenosine triphosphate (ATP) into adenosine monophosphate (AMP) and inorganic phosphate [1, 2]. This enzyme is important for helping hematophagous arthropods blood feeding, which interferes host blood coagulation or inhibiting platelet aggregation [2]. Apyrase activity depend on an ion-co factor, either calcium or magnesium [3]. ATP and ADP are important inducer to platelets aggregation, as they will interact with P2 receptors. The P2Y12 and P2Y1 are important receptor for ADP-induced platelet aggregation, while P2X1 is receptor for ATP [4]. They were released by injured cells during the hematophagous arthropoda blood feeding [1, 5]-[7].

A. Lelono et al. (Eds.): ICOLIB 2021, ABSR 27, pp. 135–143, 2023.
https://doi.org/10.2991/978-94-6463-062-6_14

Hemostasis and inflammation could be inhibited by apyrase thus the hematophagous arthropods can blood feeding easily and enhance pathogen transmission [6]. Apyrase detected in ticks, bugs and mosquitos' saliva and the other blood-feeding arthropods. It has been identified in *Ixodes dammini* [8], and *Ornithodoros moubata* [9], *Ornithodoros savignyi* [10], *Phlebotomus papatasi* [11], and *Triatoma infestans* [12] and in mosquitoes such as *Aedes aegypti* [13], *Aedes albopictus* [14] and *Anopheles gambiae* [15].

The apyrase enzyme which is expressed by gene apyrase of adult female mosquitoes has a molecular weight about 68 kDa [16]. The distal-lateral dan medial lobes of salivary gland resul apyrase involved in blood-feeding process [17, 18]. The previous studies showed that apyrase of Aedes albopictus expressed in the distal-lateral lobes was 80% and 20% of the medial lobes [19]. *Aedes albopictus*'s apyrase has molecular weight at 61 kD [20].

The previous study apyrase abundantly was detected at 56 kDa from *Aedes aegypti* salivary gland [21]. The objective of this study is to analyzed apyrase functional properties of this immunogenic protein. The apyrase activities were measured based on the amount of inorganic phosphate released from ADP degradation using malachite green detection kit and also further analyzed its platelet aggregation inhibition.

2 Materials and Methods

2.1 Rearing and Salivary Gland Dissection

In this experiment, we used *Ae. Aegypti* which was reared on laboratory scale. Rearing of *Ae. Aegypti* was carried out on laboratory scale under controlled conditions at 28 °C with 60% relative humidity at Animal Care Unit Zoology Laboratory of Biology Department, Faculty of Mathematic and Natural Sciences, University of Jember. The cage with dimension of $1 \times 1 \times 1$ m^3 for maintaining the pupae into adult mosquitos. The male adult mosquitoes were given nutrition with 10% sucrose solution and female mosquitoes feeding by fresh blood from wistar rat. The female adult mosquitoe's salivary glands were isolated by using microdissection method. Then the salivary glands were pooled into a new microtube in PBS-PMSF (Phenyl Methyl Sulfonyl Fluoride) (Sigma-Aldrich, USA) then stored frozen at -20 °C until needed.

2.2 Sodium Dedocyl Sulphate-Polyacrilamide Gel Electrophoresis (SDS-PAGE) Analysis

Protein samples were separated based on their molecular weight using the SDS-PAGE method with 12% separating gel and 4% stacking gel. Electrophoresis was performed using a constant voltage of 120 V for ±2 h at room temperature. Protein bands were visualized using Commassie Brilliant Blue (CBB) R250 (Sigma-Aldrich, USA).

2.3 Electroelution and Dialysis

The band of 56 kDa was excised using a sharp disposable blade and then electroluted using 6–8 cm cellophane membrane that already clamped to one side. Proteins were

electro-eluted for 60 min, constant voltage 120 V at room temperature. In order to remove the chemicals that were not needed, the elution procedure was continued with a dialysis process for 24 h using fresh cold PBS in a cold chamber. The buffer was replaced with a fresh one every 8 h. The protein was precipitated and concentrated overnight in a cold chamber by adding an equal volume of cold ethanol. The supernatant was centrifuged at 12.000 rpm for 15 min at 4 °C. The pellet obtained was air-dried and resuspended in 0.05 M Tris-HCl pH 6,8.

2.4 Blood Sera Collection

Sera blood samples were taken from neonates, healthy person and Dengue Hemorrhagic Fever (DHF) patient living in Jember-East Java, Indonesia. All participants gave written informed consent to take part in the study. The collecting protocol was approved by the Ethical Committee of Dentistry Faculty, University of Jember Indonesia No: 1034 / UN25.8 / KEPK / DL / 2020.

2.5 Human Immune Response by in Vitro Analysis Using ELISA

In vitro human immune response to 56 kDa from the salivary glands of the *Aedes aegypti* was measured using indirect ELISA analysis. Indirect ELISA procedure begins with antigen coating by adding 50 μL of 56 kDa protein extract into each well and was incubated overnight at 4 °C. then washed with 250 μl of PBST (Phospate Buffer Saline Tween). The next step was coating buffer blocking by adding 200 μL of blocking buffer into each well and incubating for 1 h at 37 °C, washed again with 250 μL of PBST, then added with 50 μL of primary antibody which has been diluted in blocking buffer in a ratio of 1:100 and incubated for 1 h at 37 °C. It was washed again with 250 μL of PBST and added 50 μL of secondary antibody (anti human IgG-HRP conjugated (1:5000) (Rockland, USA) then incubated for 1 h at 37°C. Enzyme activity was detected by incubating for 10 min at room temperature with 50 μL Tetra Methyl Benzidine (TMB) (Sigma-Aldrich, USA). Enzymatic reaction was stopped using 50 μL 1M H_2SO_4 for 10 min at room temperature. Optical density (OD) at 450 nm was determined with a microplate reader.

2.6 Apyrase Activity Assay

The apyrase activity was measured based on the amount of inorganic phosphate released by ATP, using malachite green colourimetric detection kit (R&D System, MN, USA Catalog Number DY996). All incubations up to the step of alkaline phosphatase activity measurements were performed as recommended by the manufacturer instructions. The 56 kDa immunogenic protein sample (0.232 mg/ml) from *Aedes aegypti* was tested in this assay.

2.7 Platelet Aggregation

The platelet aggregation ability was measured using Hamasaki methods with minor modification (5). Platelet Rich Plasma (PRP) was prepared by centrifugation of anti-coagulated blood from healthy people at 1500 rpm for 10 min at room temperature.

Platelet aggregation was determined by measuring the change in the optical density. As much as 30 µl of 56 kDa protein (0.745 µg/µl) was added with 70 µl PRP and 10 µl ADP 20 µM (Sigma-Aldrich, USA), then incubated on a shaker for 10 min. Platelet aggregation was measured by microplate reader at a wavelength of 630 nm.

3 Result and Discussion

3.1 Apyrase Activity

The 56 kDa protein induced immune responses of people living in endemic areas. Based on western blot analysis this protein was recognized by only healthy and DHF sera sample. Proteomic analysis of these protein showed that most abundant protein from 56 kDa band was apyrase [21]. Apyrase is an enzyme that inhibits platelet aggregation process and very important in the blood feeding process in arthropods hematophagous. This enzyme was able to inhibit platelet aggregation by by breaking down the phosphodiester bonds and hydrolyzes ATP or ADP into AMP and inorganic phosphate [1, 16, 22, 23].

Determination of apyrase activity measured based on the amount of inorganic phosphate released from ATP by using the malachite green colorimetric detection kit (R&D). Malachite green is a chemical dye that has various uses, its reaction with phosphomolybdate results in an intense absorbance band at 620 nm wavelength [24]. Our results showed that 56 kDa immunogenic protein had an apyrase activity of 33.30 nmol/well inorganic phosphate released. It was higher than half of positive control activity itself (ATP-se). The apyrase activity of both positive control (ATP ase) and total extract of salivary gland were 56.97 nmol/well, 35.97 nmol/well respectively (Fig. 1).

3.2 Platelet Aggregation

The human platelet aggregation by in vitro analysis was inhibited by 56 kDa protein of salivary gland of *Aedes aegypti* up to 14–70% by destroying its ADP (Fig. 2). The percentage of inhibition in the PRP sample by apyrase showed higher results than the negative control (non-treatment) and the positive control (Aspirin 0.1 mg/ml). These results indicated that the 56 kDa protein has similar activities as apyrase, which is capable in hydrolyzing ADP to inhibit platelet aggregation in human. This study results supported our hypothesis that this protein has the same ability as aspirin which is a thrombolytic agent, and thus apyrase is potentially used as a thrombolytic agent.

Salivary gland of all arthropods hematophagous including mosquitoes, sand fly, bugs or ticks have apyrase. Apyrase is nucleoside triphosphate-diphosphohydrolase

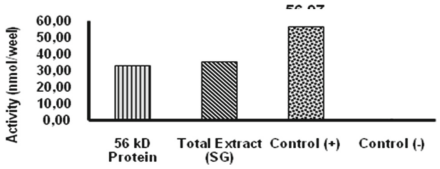

Fig. 1. Liberation of inorganic phosphate (Pi) from ATP (nmol/well) by apyrase activity.

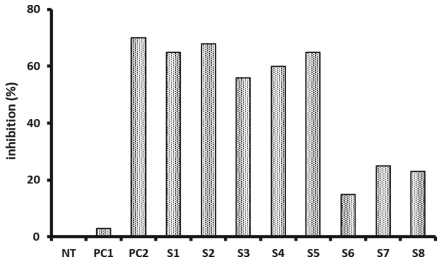

Fig. 2. Platelet aggregation inhibition. NT (negative control); PC1 PC2 (positive control Aspirin) and S1-S8 (PRP sample + 56 kD 0,1 mg/mL).

enzyme and this enzyme can hydrolyze ATP or ADP into AMP and P, so it can inhibit platelet aggregation. ADP as an inducer in the aggregation of platelet process is released from damaged cells by hematophagous arthropod bites [1, 5]. ADP is the signal for platelet aggregation, and the activities depend on the ion co-factors, both calcium, and magnesium [3].

Platelet aggregation induced by ADP can be inhibited by recombinant apyrase isolated from *Phlepotomusduboscqi* (rPduApy) by 40% without incubation and about 90% with incubation. Apyrase recombinant of *Aedes albopictus* from cloned showed a biological activity to inhibit platelet aggregation [6]. The platelet aggregation inhibition by ADP as an inducer was 8–12% [16]. Based on the ability in inhibiting platelet aggregation, apyrase was able to be developed as an anti-thrombotic protein that can be used as a potential treatment of thrombotic disease [5].

Platelet activation is induced by ADP, thrombin, and collagen molecules. Activated platelets will release mediator granules that play a role in coagulation, angiogenesis, molecular adhesion, and cytokines and chemokines. The released granules will then accelerate the activation of the platelets to form platelet aggregation [1]. This platelet activity can be inhibited by proteins produced by apyrase which is able to break down ADP thus these processes can be inhibited and facilitate mosquitoes in the blood feeding process [17].

3.3 Human Immune Response by in Vitro Analysis Using ELISA

The Elisa analysis results of cross react between 56 kDa protein salivary gland and sera human from all sample showed that the highest IgG response was detected in sera from dengue patients compared to healthy people and infants, either on individual response (Fig. 3) or pool sera response (Fig. 4).

These results indicate that people living in DHF endemic area have a spesific antibody to 56 kDa protein, and this prove that salivary proteins are immunogenic and can induce specific antibody responses [25]. They have spesific antibody due frequent exposure to saliva of mosquitoes [26]. Similiar result from another study showed that travelers in tropical country have a significant increase in antibody response to saliva mosquitoes [27].

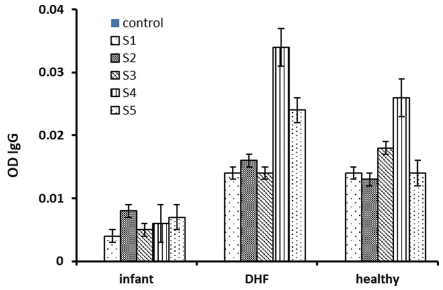

Fig. 3. Individual human immune response against protein of 56 kD.

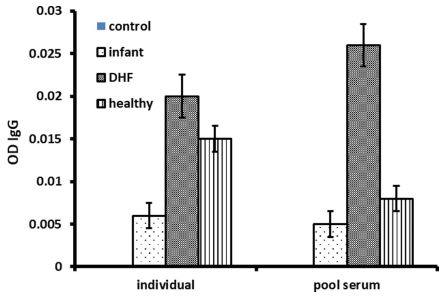

Fig. 4. Individual and pool sera human immune response against 56 kD proteins.

The concentrations of IgG as the host immune response to saliva arthropods haematophagous are correlated to the frequency of exposure [28]. Anti salivary proteins have been developed since the host exposed frequently to the mosquitoe's saliva [29]. IgG responses on DHF patient were higher than healthy people, it could be DHF patient were more exposed to *Aedes aegypti* saliva. The results of the study on malaria endemic population found that anti-saliva titers (IgG) were detected higher due to the repeated exposure to Anopheles mosquitoes [30].

A factor presence in salivary glands of *Aedes aegypti,* called 56 kDa immunogenic proteins, is believed to have apyrase activity 33.30 nmol/well, which could inhibit platelet aggregation in human plasma. The human platelet aggregation was inhibited by this immunogenic protein up to 40–50% by destroying its ADP. This protein may also elicit IgG response in humans, both from individual and pooled sera. The highest rate of IgG was detected in sera from dengue patients than those from infant and healthy people.

Acknowledgments. The authors acknowledge the financial support received from Fundamental Research Grant of Directorate General of Higher Education Indonesia, for their support and encouragement in carrying out this study.

Authors' Contributions. RO conceived and planned the experiments and wrote the manuscript. AS and EE carried out the experiment and contributed to the interpretation of the results. SW contributed to sample preparation and interpretation of the results. KS conceived the original idea and supervised the project. All authors read and approved the final manuscript.

References

1. I.M. Francischetti, Platelet aggregation inhibitors from hematophagous animals, Toxicon, 2010,vol. 56(7), pp.1130-1144. DOI: https://doi.org/10.1016/j.toxicon.2009.12.003
2. A.L. Hughes, Evolution of the salivary apyrases of blood-feeding arthropods, Gene, 2013, vol.527(1), pp.123-130. DOI: https://doi.org/10.1016/j.gene.2013.05.087
3. H.E. Reno, R.J. Novak, Characterization of apyrase-like activity in Ochlerotatustriseriatus, Ochlerotatushendersoni, and Aedes aegypti, The American journal of tropical medicine and hygiene, 2005, vol. 73(3), pp. 541-545. PMID: 16172478.
4. S.P. Kunapuli, P2 receptors and platelet activation, The Scientific World Journal, 2002, pp. 424–433. DOI: https://doi.org/10.1007/s11302-011-9247-6
5. R. Hamasaki, H. Kato, Y. Terayama, H. Iwata, J.G. Valenzuela, Functional characterization of a salivary apyrase from the sand fly, Phlebotomusduboscqi, a vector of Leishmania major, Journal of insect physiology, 2009, vol. 55(11), pp.1044-1049. DOI: https://doi.org/10.1016/j.jinsphys.2009.07.010
6. G. Caljon, K. De Ridder, P. De Baetselier, M. Coosemans, J. Van Den Abbeele, Identification of a tsetse fly salivary protein with dual inhibitory action on human platelet aggregation, PLoS One, 2010, vol. 5(3). DOI: https://doi.org/10.1371/journal.pone.0009671
7. A. Fontaine, I. Diouf, N. Bakkali, D. Missé, F. Pagès, T. Fusai, L. Almeras,Implication of haematophagous arthropod salivary proteins in host-vector interactions, Parasites & vectors, 2011, vol. 4(1), pp.187. DOI: https://doi.org/10.1186/1756-3305-4-187
8. J.M. Ribeiro, G.T. Makoul, J. Levine, D.R. Robinson, A. Spielman,Antihemostatic, antiinflammatory, and immunosuppressive properties of the saliva of a tick, Ixodes dammini, The Journal of experimental medicine, 1985, vol. 1(2), pp. 332-344. DOI: https://doi.org/10.1084/jem.161.2.332
9. J.C. Ribeiro, T.M. Endris, R. Endris, Saliva of the soft tick, Ornithodorosmoubata, contains anti-platelet and apyrase activities, Comparative Biochemistry and Physiology Part A: Physiology, 1991, vol. 100(1), pp. 109-112. DOI: https://doi.org/10.1016/0300-9629(91)90190-n
10. B.J. Mans, A.R.M.D. Gaspar, A.I.Louw, A. A.W.H. Neitz,Apyrase activity and platelet aggregation inhibitors in the tick Ornithodorossavignyi (Acari: Argasidae),Experimental & applied acarology,1998, vol. 22(6), pp. 353-366. DOI: https://doi.org/10.1023/a:1024517209621
11. J.G. Valenzuela, Y.A. Belkaid, E.D.Rowton, J.M. Ribeiro,Thesalivary apyrase of the blood-sucking sand fly Phlebotomuspapatasi belongs to the novel Cimex family of apyrases, Journal of Experimental Biology, 2001, vol. 204(2), pp. 229-237. DOI: https://doi.org/10.1242/jeb.204.2.229
12. E. Faudry, S.P. Lozzi, J.M. Santana, M. D'Souza-Ault, S. Kieffer, C.R. Felix, A.R. Teixeira,Triatoma infestans apyrases belong to the 5′-nucleotidase family, Journal of Biological Chemistry, 2004, vol. 279(19), pp. 19607-19613. DOI: https://doi.org/10.1074/jbc.M401681200
13. D.E. Champagne, C.T. Smartt, J.M. Ribeiro, A.A. James,Thesalivary gland-specific apyrase of the mosquito Aedes aegypti is a member of the 5'-nucleotidase family, Proceedings of the National Academy of Sciences, 1995, vol. 92(3), pp. 694-698. DOI: https://doi.org/10.1073/pnas.92.3.694

14. B. Arca, F. Lombardo, I.M. Francischetti, V.M. Pham, M. Mestres-Simon, J.F. Andersen, J.M. Ribeiro, An insight into the sialome of the adult female mosquito Aedes albopictus,Insect biochemistry and molecular biology, 2007, vol. 37(2), pp. 107-127. DOI: https://doi.org/10.1016/j.ibmb.2006.10.007
15. F. Lombardo, M. Di Cristina, L. Spanos, C. Louis, M. Coluzzi, B. Arcà, Promoter sequences of the putative Anopheles gambiae apyrase confer salivary gland expression in Drosophila melanogaster, Journal of Biological Chemistry, 2000, vol. 275(31), pp. 23861-23868. DOI: https://doi.org/10.1074/jbc.M909547199
16. F. Dong, Y. Fu, X. Li, J. Jiang, J.Sun, X. Cheng, Cloning, expression, and characterization of salivary apyrase from Aedes albopictus, Parasitology research, 2012, vol. 110(2), pp. 931-937. DOI: https://doi.org/10.1007/s00436-011-2579-x
17. C.T. Smartt, A.P. Kim, G.L. Grossman, A.A. James,The Apyrase Gene of the Vector Mosquito, Aedes eegypti, Is Expressed Specifically in the Adult Female Salivary Glands, Experimental parasitology, 1995, vol. 81(3), pp. 239-248. DOI:https://doi.org/10.1006/expr.1995.1114
18. J. Juhn, U. Naeem-Ullah, B.A.M. Guedes, A. Majid, J. Coleman, P.F.P. Pimenta, O. Marinotti,Spatial mapping of gene expression in the salivary glands of the dengue vector mosquito, Aedes aegypti, Parasites & vectors, 2011, vol. 4(1), pp. 1-13. DOI: https://doi.org/10.1186/1756-3305-4-1
19. P.A. Rossignol, J.M.C. Ribeiro, A. Spielman,Increased intradermal probing time in sporozoite-infected mosquitoes, The American journal of tropical medicine and hygiene, 1984, vol. 33(1), pp. 17-20. DOI: https://doi.org/10.4269/ajtmh.1984.33.17
20. O. Marinotti, M. de Brito, C.K. Moreira,Apyrase and α-glucosidase in the salivary glands of Aedes albopictus, Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 1996. vol. 113(4), pp. 675-679. DOI: https://doi.org/10.1016/0305-0491(95)02035-7
21. R. Oktarianti, K. Senjarini, T. Hayano, F. Fatchiyah,Proteomic analysis of immunogenic proteins from salivary glands of Aedes aegypti, Journal of Infection and PublicHealth, 2015, vol. 8(6), pp. 575-582. DOI: https://doi.org/10.1016/j.jiph.2015.04.022
22. J.M. Ribeiro, I.M. Francischetti, Role of arthropod saliva in blood feeding: sialome and post-sialome perspectives, Annual review of entomology, 2003, vol. 48(1), pp. 73-88. DOI: https://doi.org/10.1146/annurev.ento.48.060402.102812
23. N. Jariyapan, S. Roytrakul, A. Paemanee, A. Junkum, A. Saeung, S. Thongsahuan, W. Choochote, Proteomic analysis of salivary glands of female Anopheles barbirostris species A2 (Diptera: Culicidae) by two-dimensional gel electrophoresis and mass spectrometry, Parasitology research, 2012, vol. 111(3), pp. 1239-1249. DOI: https://doi.org/10.1007/s00436-012-2958-y
24. K. Itaya, M. Ui,A new micromethod for the colorimetric determination of inorganic phosphate, Clinicachimica acta, 1996, vol. 14(3), pp. 361-366. DOI: https://doi.org/10.1016/0009-8981(66)90114-8
25. F. Remoue, E. Alix, S. Cornelie, C. Sokhna, B. Cisse, S. Doucoure, F. Simondon,IgE and IgG4 antibody responses to Aedes saliva in African children, Acta tropica, 2007, vol. 104(2-3), pp. 108-115. DOI: https://doi.org/10.1016/j.actatropica.2007.07.011
26. S. Cornelie, F. Remoue, S. Doucoure, T. NDiaye, F.X. Sauvage, D. Boulanger, F. Simondon, An insight into immunogenic salivary proteins of Anopheles gambiae in African children, Malaria journal, 2007, vol. 6(1), pp. 1–7. DOI: https://doi.org/10.1186/1475-2875-6-75
27. E. Orlandi-Pradines, L. Almeras, L.D. de Senneville, S. Barbe, F. Remoué, C. Villard, C. Rogier,Antibody response against saliva antigens of Anopheles gambiae and Aedes aegypti in travellers in tropical Africa, Microbes and infection, 92007, vol. (12–13), pp. 1454–1462. DOI: https://doi.org/10.1016/j.micinf.2007.07.012

28. Z. Peng, F.E.R. Simons, 2004. Mosquito allergy: immune mechanisms and recombinant salivary allergens, International archives of allergy and immunology, 2004, vol. 133(2), pp. 198-209. DOI: https://doi.org/10.1159/000076787
29. S. Doucouré, S.Cornélie, S.Patramool, F. Mouchet, E. Demettre, M. Seveno, F. Remoué, First screening of Aedes albopictus immunogenic salivary proteins, Insect molecular biology, 2013, vol. 22(4), pp. 411-423. DOI: https://doi.org/10.1111/imb.12032
30. A. Waitayakul, S. Somsri, J. Sattabongkot, S. Looareesuwan, L. Cui, R. Udomsangpetch, Natural human humoral response to salivary gland proteins of Anopheles mosquitoes in Thailand, Acta tropica, 2006, vol. 98(1), pp. 66-73. DOI: https://doi.org/10.1016/j.actatropica.2006.02.004